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PROVISIONAL PATENT APPLICATION COVER SHEET

This is a request for filing a PROVISIONAL APPLICATION under 37 CFR 1.53(b)(2).

Docket Number	7230-21	Type a plus sign (+) inside this box =>	+

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TITLE OF THE INVENTION (230 characters max)

TOPICAL CO-ENZYME Q10 FORMULATIONS AND METHODS OF USE

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Respectfully submitted,

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TOPICAL CO-ENZYME Q10 FORMULATIONS AND METHODS OF USE

FIELD OF THE INVENTION

The invention relates generally to the fields of pharmacy and medicine. More particularly, the invention relates to topical preparations of co-enzyme Q10 (CoQ10) and methods of using CoQ10 for selectively reducing cancer cell growth.

BACKGROUND

Cancer is presently one of the leading causes of death in developed nations. Although recent research has vastly increased our understanding of many of the molecular mechanisms of tumorigenesis and has provided numerous new avenues for the treatment of cancer, standard treatments for most malignancies remain gross resection, chemotherapy, and radiotherapy. While increasingly successful, each of these treatments still causes numerous undesired side effects. For example, surgery results in pain, traumatic injury to healthy tissue, and scarring. Radiotherapy and chemotherapy cause nausea, immune suppression, gastric ulceration and secondary tumorigenesis.

SUMMARY

The invention relates to the discovery that topical formulations of CoQ10 can reduce the rate of tumor growth in an animal subject. In the experiments described herein, CoQ10 was shown to increase the rate of apoptosis in a culture of skin cancer cells but not normal cells. Moreover, treatment of tumor-bearing animals with a topical formulation of CoQ10 was shown to dramatically reduce the rate of tumor growth in the animals.

CoQ10 formulated for oral delivery has previously been used as a dietary supplement. Orally administered CoQ10 has, however, been shown to accumulate in the liver- diminishing its systemic availability. The anti-tumor responses observed with topically applied CoQ10 may relate to its higher bioavailability compared to dietary supplement forms of the CoQ10.

Accordingly, the invention features a method for reducing the rate of tumor cell growth or increasing the rate of apoptosis in tumor cells in a subject. The method includes the steps of providing a subject having a plurality of tumor cells and administering to the subject a composition comprising an effective amount of CoQ10 and a pharmaceutically acceptable carrier.

In another aspect, the invention features a composition comprising an effective amount of CoQ10 and a pharmaceutically acceptable carrier. A preferred composition is a topical formulation of CoQ10 that includes at least about 7.5% by weight CoQ10 and a carrier suitable for delivering the CoQ10 topically.

Unless otherwise defined, all technical terms used herein have the same meanings as commonly understood by one of ordinary skill in the art to which this invention belongs. Commonly understood definitions of medical terms can be found in Thomas Lathrop Stedman, Stedman's Medical Dictionary, Lippincott, Williams & Wilkins: Philadelphia, PA, 2000.

All publications, patent applications, patents and other references mentioned herein are incorporated by reference in their entirety. In the case of conflict, the present specification, including definitions will control. The particular embodiments discussed below are illustrative only and not intended to be limiting.

BRIEF DESCRIPTION OF THE DRAWINGS

Fig 1. is a series of photomicrographs showing the effect of CoQ10 on human melanoma cells (SKMEL28) in an in vitro culture.

Fig. 2 is a graph showing that CoQ10 reduces the proliferation of a human melanoma cell line (SKMEL28) in a 36 hour in vitro culture.

Fig. 3 is a graph showing that CoQ10 reduces the proliferation of a human melanoma cell line (SKMEL28) in a 48 hour in vitro culture.

Fig. 4 is a graph showing that the vehicle control does not reduce the proliferation of a human melanoma cell line (SKMEL28) in a 48 hour in vitro culture.

Fig. 5 is a graph comparing the effect of CoQ10 on apoptosis between human melanoma and neonatal fibroblasts in an in vitro culture.

Fig. 6 is a graph showing that CoQ10 reduces the proliferation of squamous carcinoma cells in a 48 hour in vitro culture.

Fig. 7 is a graph showing that CoQ10 reduces the proliferation of human neonatal fibroblasts in a 48 hour in vitro culture.

Fig. 8 is a graph showing that CoQ10 increases the proliferation of human neonatal keratinocytes in a 48 hour in vitro culture.

Fig. 9 is a graph showing that CoQ10 reduces the proliferation of a breast adenocarcinoma cell line (MCF-7) in a 48 hour in vitro culture.

Fig. 10 is a graph showing that CoQ10 reduces the proliferation of a breast adenocarcinoma cell line (MCF-7) in a 72 hour in vitro culture.

Fig. 11 is a photograph showing induced tumors in control and CoQ10-treated mice after treatment with topical formulation of CoQ10 for 30 days.

Fig. 12 is a photograph showing induced tumors in control and CoQ10-treated mice after treatment with topical formulation of CoQ10 for 30 days.

Fig. 13 is a photograph showing tumors excised from control and CoQ10-treated mice.

Fig. 14 is a graph showing the effect of CoQ10 administration on tumor size in mice treated with CoQ10 or control for 30 days.

DETAILED DESCRIPTION

The invention provides compositions and methods for reducing the rate of tumor cell growth or increasing the rate of tumor cell apoptosis. Compositions of the invention include as an anti-tumor agent an effective concentration of CoQ10 and a carrier. A preferred composition of the invention is a topical formulation of CoQ10 that contains at least about 7.5% CoQ10 and a carrier that facilitates topical delivery of CoQ10. A most preferred composition of the invention is a topical formulation of CoQ10 that contains between about 10 and 15% CoQ10 and a carrier that facilitates topical delivery of CoQ10. Methods of the invention for killing a tumor cell or reducing its growth rate include the step of contacting the cell with an effective concentration of CoQ10.

The below described preferred embodiments illustrate adaptations of these compositions and methods. Nonetheless, from the description of these embodiments, other aspects of the invention can be made and/or practiced based on the description provided below.

CoQ10 Formulations for Treating Cancer

The invention features CoQ10-containing compositions for the treatment of cancer. In the representative embodiment described in the Examples section below, a topical formulation of CoQ10 is applied to the skin of a tumor-bearing animal to reduce the growth rate of the tumor. CoQ10 can be obtained from Pure Prescriptions (San Diego, CA) in powdered form in any suitable quantity (e.g., 1 kilogram).

To deliver a CoQ10-containing composition, any suitable carrier can be used. Liposomes, for example, may be used as a carrier. An exemplary liposomal formulation is composed of Phospholipon 90G (American Lechitin, Stanford, CT), Glycerol, Butylated

hydroxytoluene (BHT), Ethanol, Medium Chain Tryglycerides (MCT), lavender (Sigma-Aldrich, St. Louis, MO) and Coenzyme Q10 (Pure Prescriptions, San Diego, CA). An example of a protocol for preparing this formulation entails first dissolving 6g of Phospholipon 90G with 5.8g of Glycerol, 0.2g BHT, 4ml ethanol, and 18g MCT at 60°C. Next, 20g of Coenzyme Q10 are dissolved into the mixture. 90ml of 1mM phosphate buffer (pH 8.2) prepared with nitrogen saturated water and 0.2ml of lavender are added. The above mixture is blended in a high-speed blender at 12,000 PRM to form a cream. The cream is stored at 4°C until used.

Subjects

Because subjects from many different species have tumors and are susceptible to acquiring a tumor, the invention is believed to be compatible with many types of animal subjects. A non-exhaustive exemplary list of such animals includes mammals such as mice, rats, rabbits, goats, sheep, pigs, horses, cattle, dogs, cats, and primates such as monkeys, apes, and human beings. Those animal subjects known to suffer from a skin cancer tumor are preferred for use in the invention. In particular, human patients suffering from a skin cancer tumor or other tumor are suitable animal subjects for use in the invention. In the experiments described herein, the subject used were mice. Nonetheless, by adapting the methods taught herein to other methods known in medicine or veterinary science (e.g., adjusting doses of administered substances according to the weight of the subject animal), the compositions utilized in the invention can be readily optimized for use in other animals.

Pharmaceutical Compositions and Administration to a Subject

The compositions described above may be administered to a subject in any suitable formulation. In addition to treatment of cancer with topical formulations of CoQ10, in other aspects of the invention CoQ10 might be delivered by other methods. For example, CoQ10 might be formulated for parenteral delivery, e.g., for subcutaneous, intravenous, intramuscular, or intratumoral injection. Other methods of delivery, for example, liposomal delivery or diffusion from a device impregnated with the composition might be used. The compositions may be administered in a single bolus, multiple injections, or by continuous infusion (for example, intravenously or by peritoneal dialysis). For parenteral administration, the compositions are preferably formulated in a sterilized pyrogen-free form. Compositions of the invention can also be administered in vitro to a cell (for example, to induce apoptosis in a cancer

cell in an in vitro culture) by simply adding the composition to the fluid in which the cell is contained.

Effective Amounts

The compositions described above are preferably administered to a subject in an effective amount. An effective amount is an amount which is capable of producing a desirable result in a treated animal or cell (for example, to induce apoptosis or impair mitosis in a cell in the animal or a culture). As is well known in the medical and veterinary arts, dosage for any one animal depends on many factors, including the particular animal's size, body surface area, age, the particular composition to be administered, time and route of administration, general health, and other drugs being administered concurrently. It is expected that an appropriate dosage for topical administration of the compositions of the invention would be in the range of about 1.5 - 4.0 mg CoQ10/kg of body weight (e.g., 200 mg for subjects ranging from 110 to 300 lbs). An effective amount for use with a cell in culture will also vary, but can be readily determined empirically (for example, by adding varying concentrations to the cell and selecting the concentration that best produces the desired result). It is expected that an appropriate concentration would be in the range of about 5 - 200 μM.

Method for Inhibiting Cancer Cell Growth

The invention provides a method for reducing the rate of tumor cell growth or increasing the rate of tumor cell apoptosis. The method includes the steps of contacting a tumor cell with a composition including a sufficient amount of CoQ10 to kill or at least retard mitosis in the tumor cell. The method may be used to inhibit the growth of numerous types of cancerous tumor cells. Coenzyme Q10 has been tested and shown to be effective against melanoma, squamous, and breast cancer cells. Coenzyme Q10 is expected to be effective against other cancers as well, particularly those derived from mesenchymal and hemopoietic origins.

Any suitable formulation of CoQ10 can be used in methods of the invention. Typical formulations are topical liposomal formulations of coenzyme Q10 of varying concentrations. In addition to topical administration, CoQ10 –containing formulations can be administered to a subject via injection (e.g., IP, IV, IM, SQ).

In a method of reducing the rate of tumor cell growth or increasing the rate of tumor cell apoptosis in vitro, CoQ10 is dissolved in 2-propanol followed by dilution in a desired medium (as described in example 1 below). In an in vivo method of reducing the rate of tumor cell

growth or increasing the rate of tumor cell apoptosis, a CoQ10-containing cream is applied topically daily to the target site until tumor regression occurs (as described in examples 2 and 3). In another in vivo method, a CoQ10-containing formulation is administered to a subject via injection (e.g., IP, IV, IM, SQ).

Kits

The invention also provides a kit for reducing the rate of tumor growth in a subject. The kit of the invention includes a composition comprising CoQ10 and a pharmaceutically acceptable carrier as well as printed instructions for using the composition to reduce the rate of tumor growth in a subject.

Examples

Example 1- Materials and Methods For Apoptosis Assay

Cell lines used in the assay were SK-Mel28, nFIB, and MCF-7. Cells (SK-Mel28, FIB, and MCF-7) were seeded (5 X 10^4 cells/well) into wells containing either solely medium or medium with treatment and placed in an incubator at 37°C, 5% CO_2, and under humidified conditions for 48 hours. Each condition was performed in duplicate and was subjected to the following protocol:

Apoptosis analysis as per protocol of BD Pharmingen Annexin-VPE Protocol

Reagents include Annexin V-PE (BD Pharmingen, San Diego, CA), 7-AAD (BD Pharmingen, San Diego, CA), binding buffer (10x: 0.1 M Hepes/NaOH, 1.4 M NaCl, 25 mM $CaCl_2$) [diluted to 1x (9mL PBS and 1mL binding buffer) for use in experiment] (BD Pharmingen, San Diego, CA), Trypsin-EDTA (Gibco, Grand Island, NY), and desired Media.

Add .5mL trypsin to each well, remove trypsin after approximately 10 seconds, and add .5mL trypsin to each well. Place wells in an incubator, observe level of detachment under microscope after 4 minutes, and gently tap sides and bottom to aid in detachment. When cells detach, neutralize with .5mL serum-supplemented medium. Transfer cell solution to centrifuge tubes, centrifuge cells at 2000 RPM for 5 minutes, aspirate supernatant, resuspend in 6mL PBS, and split 6mL into three centrifuge tubes (2mL each). Centrifuge cells at 2000 RPM for 5 minutes, aspirate supernatant, resuspend in 100µL binding buffer mix, add 50µL of Annexin V-PE and 50µL of 7-AAD in each centrifuge tube, and vortex and place in the dark for 15 minutes. Add 350µL binding buffer to each tube and perform analysis using the flow cytometer.

A baseline was also created using freshly cultured cells from a flask. The cells were subcultured and washed twice with cold PBS. Subsequently, they were resuspended in 1x binding buffer to a concentration of 1 X 10^6 cells/mL. 100µL of cell suspension were transferred into three test tubes for a total of 1 X 10^5 per tube. One tube served as a negative control with no staining introduced. Another was stained with only Annexin V-PE while the final was stained with only 7-AAD. 50µL of staining solution was placed into each of the tubes. These tubes were then placed in the dark for 15 minutes after which time, 350µL of binding buffer were added to each. They were then subjected to analysis by flow cytometry prior to the treated and control cells.

Experiment 1:

The Effect of Coenzyme Q10 on the level of Apoptosis in Human Breast Cancer Cells

MCF-7 Control	MCF-7 Control	MCF-7 Control
100 µM CoQ10	100 µM CoQ10	100 µM CoQ10

-Seeded 50,000 cells/well

-Compare to Baseline of 100,000 cells/sample in Apoptosis Assay (Annexin PI) after 72 hrs

Experiment 2:

The Effect of 2-Propanol Vehicle on the level of Apoptosis in Melanoma Cells

SK-MEL 28 Control	SK-MEL 28 Control	SK-MEL 28 Control
Equivalent Vol. if 50 µM of CoQ10 (1% 2-Propanol)	Equivalent Vol. if 50 µM of CoQ10 (1% 2-Propanol)	Equivalent Vol. if 50 µM of CoQ10 (1% 2-Propanol)

-Seeded 50,000 cells/well

-Compare to Baseline of 100,000 cells/sample in Apoptosis Assay (Annexin PI) after 48 hrs

Experiment 3:

The Effect of 2-Propanol Vehicle on the level of Apoptosis in Neonatal Fibroblasts

nFIB (P) 6 Control	nFIB (P) 6 Control	nFIB (P) 6 Control
Equivalent Vol. if 50 µM of CoQ10 (1% 2-Propanol)	Equivalent Vol. if 50 µM of CoQ10 (1% 2-Propanol)	Equivalent Vol. if 50 µM of CoQ10 (1% 2-Propanol)

-Seeded 50,000 cells/well

-Compare to Baseline of 100,000 cells/sample in Apoptosis Assay (Annexin PI) after 48 hrs

Preparation of DMEM/F12 Medium

Materials:

- DMEM/F12 medium (Cat# 11330-032 Gibco-Invitrogen Corp, Grand Island, NY)
- Siliconized Sterile Pipette tips – 1mL and 25mL to be used with PipettMan
- FBS (Fetal Bovine Serum) Supplement (Gibco-Invitrogen Corp,Grand Island, NY)
- PSA (Penicillin Streptomycin Amphoterocin B)- Antimicrobrial Agent Supplement (Cascade Biologics, Inc., Portland, OR)

Procedures:

Transfer appropriate amount of FBS into DMEM/F12 (e.g., 50 mL FBS in 500mL medium for 10% serum concentration). Add appropriate amount of PSA to obtain a solution with a final concentration of 100U/mL Penicillin G, 100µg/mL streptomycin sulfate, and 0.25µg/mL Amphotericin B (e.g., 1 mL of 500x PSA in 500mL medium). Mix by pipetting and inverting bottle. Store at 4°C until use.

Preparation of EpiLife Medium

Materials:

- Siliconized Sterile Pipette tips- 5mL, 10mL to be used with PipettMan
- EpiLife Media (M-EPI-500, Cascade Biologicals)
- PSA (500X Penicillin Streptomycin Amphoterocin B)- Antimicrobrial Agent Supplement (R-004-10 Cascade Biologics)

- EDGS (Epidermal Growth Supplement) (S-012-5 Cascade Biologics)

Procedures:

Transfer one vial of EDGS (5mL) and PSA (1mL) into EpiLife Medium resulting in 100U/mL Penicillin G, 100µg/mL streptomycin sulfate, and 0.25µg/mL Amphotericin B (e.g. 1 mL of 500x PSA in 500mL medium). Mix by pipetting and inverting. Store in 4°C until use

Creating a Homogenous Solution of Q10 in Media Protocol

Materials:

- Polystyrene Sterile Pipette tips- 200-1000 µM to be used with automatic pipettes
- Siliconized Sterile Pipette tips- 10mL to be used with PipettMan
- 15 mL Centrifuge Tubes
- Media
-Coenzyme Q10 (Compound Solutions, Inc., Escondido, CA)
-2-propanol (Cat# 9083-3, J.T. Baker Chemical Co., Phillipsbury, NJ)

Procedures:

Retrieve Q10 stock from –20°C storage and weigh out approximately 4.4 mg. Transfer Q10 into a 25mL centrifuge tube. Add 1mL 2-propanol to centrifuge tube. Vortex and dip in hot water bath (55°C) to promote dissolution. Add 9mL of media to centrifuge tube. Vortex and dip in hot water bath (55°C) if necessary to create a homogenous solution. This results in a 500 µM Q10 solution. Make serial dilutions to treatment concentrations

Defrosting Cells Protocol

Materials:

- Siliconized Sterile Pipette tips- 1mL, 10mL to be used with PipettMan
- 75cm^2 Cell Culture Flasks
- 15 mL Centrifuge Tubes

Procedures:

Acclimate reagents to 37°C in water bath. Remove cells from liquid nitrogen tank. Keep vial clasped in palm to initiate defrost. Submerge in water bath at 37°C until completely melted. Transfer cells to a 15mL centrifuge tube. Mix by pipetting. Centrifuge at 2500 RPM for 8 minutes. Aspirate supernatant. Resuspend pellet with appropriate medium. Mix by vortexing and pipetting to homogenize cell suspension. Transfer to 75cm^2 Cell Culture flask(s) .

Subculturing Cells Protocol

Materials:

- Siliconized Sterile Pipette tips- 5mL, 10mL to be used with PipettMan
- 75cm2 (T75) Cell Culture Flasks
- 6 Well Tissue Culture Plates
- 15mL Centrifuge Tubes
- Media
- 0.05% Trypsin (Cat# 25-052-CI- 1X Trypsin-EDTA, Cellgro by Mediatech, Herndon, VA)

Procedures:

Acclimate reagents to 37°C in water bath. Remove medium from cell culture flasks (cells are ready for subculture when approximately 85% confluent). Prime by adding 1-2mL of trypsin to flask for 30 seconds. Remove trypsin from flask. Add 5mL of trypsin to flask. Place flask in the incubator at 37°C for approximately 4 minutes. Remove and observe degree of detachment with microscope. If needed, gently tap flask to aid in detachment. Add 5mL of serum-containing medium. Mix by pipetting and washing flask with cell suspension. Transfer cell suspension to a 15mL centrifuge tube. Vortex centrifuge tube. Centrifuge at 2500 RPM for 8 minutes. Aspirate supernatant. Resuspend pellet in appropriate medium. Create a homogenous cell suspension by pipetting and vortexing. Seed cells in new T75 flasks or into wellplates for experimentation.

Counting Cells Protocol

Materials:

-Beckman Coulter® Z1 Cell and Particle Counter (Beckman Coulter, Inc., Fullerton, CA)
-Coulter Counter Vials (Beckman Coulter, Inc.)
-Isoton II Diluent (# 8546719, Beckman Coulter)
-Coulter CLENZ (# 8546929, Beckman Coulter)
- Polystyrene Sterile Pipette tips- 20-200 μM, 200-1000 μM to be used with automatic pipettes

Procedures:

After subculture (per subculturing cells protocol described above), pipet the desired volume suspension of cells to count (.25-1mL) into Coulter Counter Vial (Beckman, Inc.) using an automatic pipette. Insure that the Beckman Coulter® Z1 Cell and Particle Counter is clean by using Coulter CLENZ (Beckman, Inc., Fullerton, CA) to flush. Flush apparatus once with Isoton

II Diluent. Add Isoton II Diluent to vial containg cells for a total volume of 10mL. Use output mode of apparatus to count cells twice to ensure accuracy. Average counts together and calculate total cell number per volume.

Performing in vitro Experiments Protocol

Materials:

- Polystyrene Sterile Pipette tips- 20-200 µM, 200-1000 µM to be used with automatic pipettes
- Siliconized Sterile Pipette tips- 5mL, 10mL to be used with PipettMan
- 75cm2 Cell Culture Flasks
- 6 Well Tissue Culture Plates
- 15 mL Centrifuge Tubes
- Coulter Counter Vials (Beckman Coulter, Inc.)
- 0.05% Trypsin (Cat# 25-052-C1- 1X Trypsin-EDTA, Cellgro)

Procedures:

Acclimate reagents to 37°C in water bath. Make stock solution of Q10 as per protocol described above for creating a homogenous solution of Q10 in media. Perform serial dilutions to desired concentrations. Place 2mL media into respective wells. Subculture flasks as per protocol described above for subculturing cells. Resuspend cells with just enough medium to create a homogenous cell suspension (approximately 5mL). Determine cell concentration as per protocol described above for counting cells. Dilute cell suspension so that the desired amount of cells to seed is contained within 50-100µL. Seed desired amount of cells into each well. Incubate at 37°C, 5% CO_2, and under humidified conditions for desired duration. Aspirate media from wells. Place .5mL trypsin into each well. Incubate for approximately 4 minutes. Check for degree of detachment under microscope. Swirl, gently tap sides, and gently knock bottom to aid in detachment if necessary. Neutralize trypsin with .5mL medium. Pipette to aid in cell detachment and breaking of clumps. Remove .5mL cell suspension and place in coulter counter vials (Beckman Coulter, Inc.). Count cells as per protocol described above for counting cells.

Inoculation of Animals Protocol

Materials:

- Phospate buffer solution (PBS) (Gibco-Invitrogen Corp,Grand Island, NY)
- Polystyrene Sterile Pipette tips- 20-200 µM, 200-1000 µM to be used with automatic pipettes
- Siliconized Sterile Pipette tips- 5mL, 10mL to be used with PipettMan

- 75cm^2 Cell Culture Flasks
- 15 mL Centrifuge Tubes
- Coulter Counter Vials (Beckman Coulter Inc.)
- 0.05% trypsin (Cat# 25-052-C1- 1X Trypsin-EDTA, Cellgro)
- Centrifuge tubes (2mL)
- Anesthetic (Aventin)

Procedures:

Subculture flasks as per the cell subculturing protocol described above. After aspirating supernatant, combine pellets from each flask diluted slightly with PBS with a 5mL pipette. Dilute final cell suspension to contain approximately ten million cells per 100µL. Transfer cell suspension to micro-centrifuge tubes (2mL). Place in ice immediately and leave in ice until injected. Anesthetize mice via an intraperitoneal injection with 0.3cc Aventin. Inoculate each animal subcutaneously with 0.1cc cell suspension per site. Transfer any remaining cells into a 15mL centrifuge tube. Dilute to 10mL with medium. Centrifuge at 2500 RPM for 8 minutes. Aspirate supernatant. Add 10mL media to centrifuge tube. Create a homogenous cell suspension by pipetting and vortexing. Seed cells in a T75 flask to ensure experimental cell viability.

Example 2 – Effect of A Topical Formulation Of Coenzyme Q10 on SK-28 Tumors In Mice

Melanoma tumors were induced in mice by SK-MEL28 injection into the subcutaneous layer. The animal study consisted of both a control and treatment group each containing four mice. The mice were inoculated with two tumors and the graph of Figure 14 represents the resulting mean mass for the tumors in each mouse. A topical formulation of Coenzyme Q10 (10%) was applied to the tumors in the treatment group daily for a period of 30 days. After which, the tumors were excised and the mass was determined. The difference in the overall mean mass of the treatment group was significant compared to the control ($P<0.05$).

Example 3-Preparation of Topical CoQ10 Cream

Reagents:

-Phospholipon 90G (American Lechitin, Stanford, CT)
-Glycerol
-BHT
-Ethanol

-MCT

-lavender (Sigma-Aldrich)

-CoQ10 (Pure Prescriptions, San Diego, CA)

Procedure:

6g of Phospholipon 90G (American Lechitin, Stanford, CT) was dissolved in a mixture of 5.8g of Glycerol (Sigma-Aldrich, St. Louis, MO), 0.2g BHT (Sigma-Aldrich), 4ml ethanol (Sigma-Aldrich), and 18g MCT (Sigma-Aldrich) at 60°C. 20g of CoQ10 (Pure Prescriptions) were dissolved into the resulting mixture. 90ml of 1mM phosphate buffer (pH 8.2) prepared with nitrogen saturated water and 0.2ml of lavender (Sigma-Aldrich) were added and the mixture was blended in a high speed blender at 12,000 RPM to form a cream. The cream was stored at 4°C until used.

Other Embodiments

It is to be understood that while the invention has been described in conjunction with the detailed description thereof, the foregoing description is intended to illustrate and not limit the scope of the invention, which is defined by the scope of the appended claims. Other aspect, advantages, and modifications are within the scope of the following claims.

What is claimed is:

1. A composition comprising CoQ10 and a pharmaceutically acceptable carrier.

2. A method for reducing the rate of tumor cell growth or increasing the rate of apoptosis in tumor cells in a subject, the method comprising the steps of:

(A) providing a subject having a plurality of tumor cells; and

(B) administering to the subject a composition comprising CoQ10 and a pharmaceutically acceptable carrier.

ABSTRACT

Topical formulations of CoQ10 reduce the rate of tumor growth in an animal subject. In the experiments described herein, CoQ10 was shown to increase the rate of apoptosis in a culture of skin cancer cells but not normal cells. Moreover, treatment of tumor-bearing animals with a topical formulation of CoQ10 was shown to dramatically reduce the rate of tumor growth in the animals.

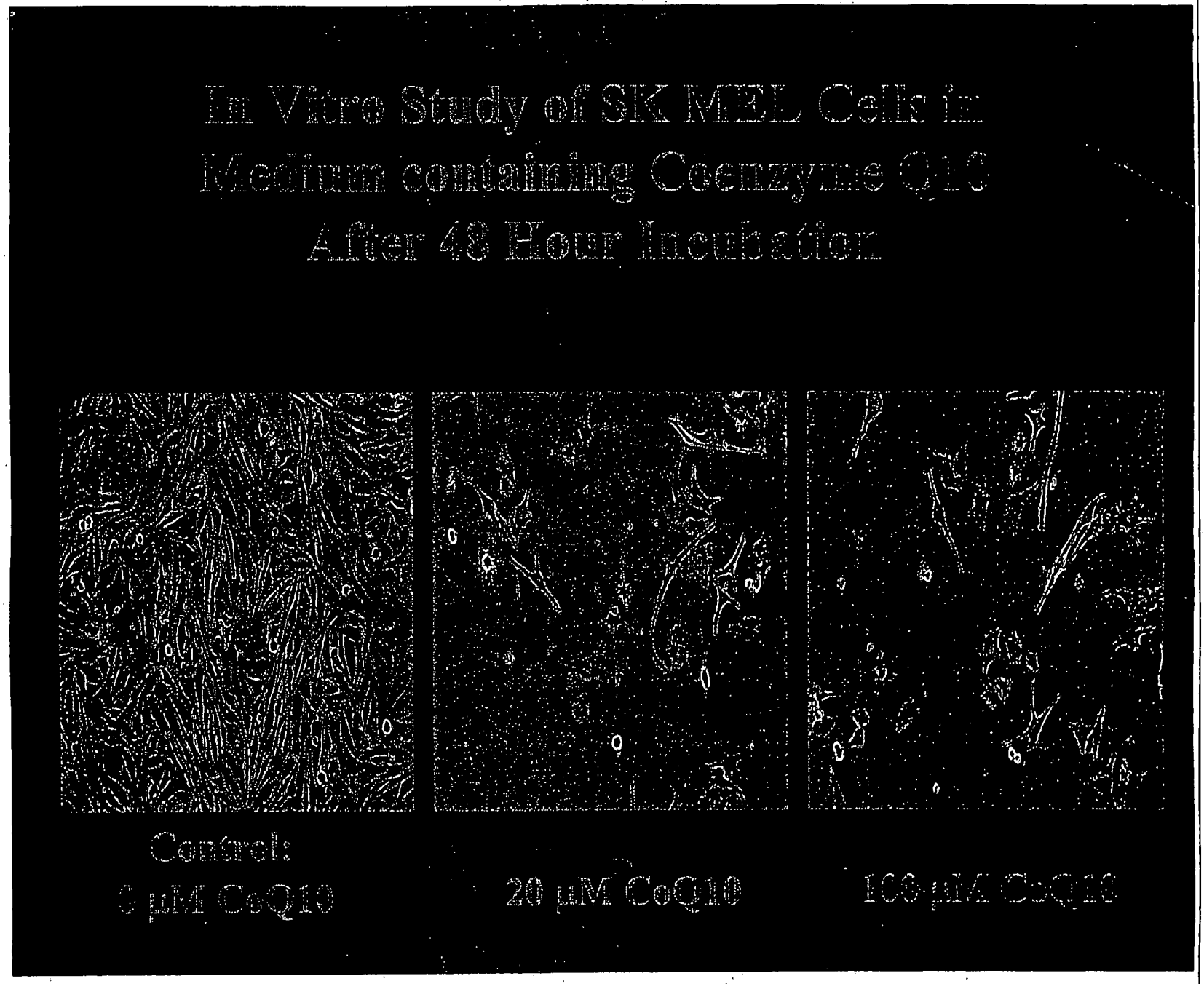
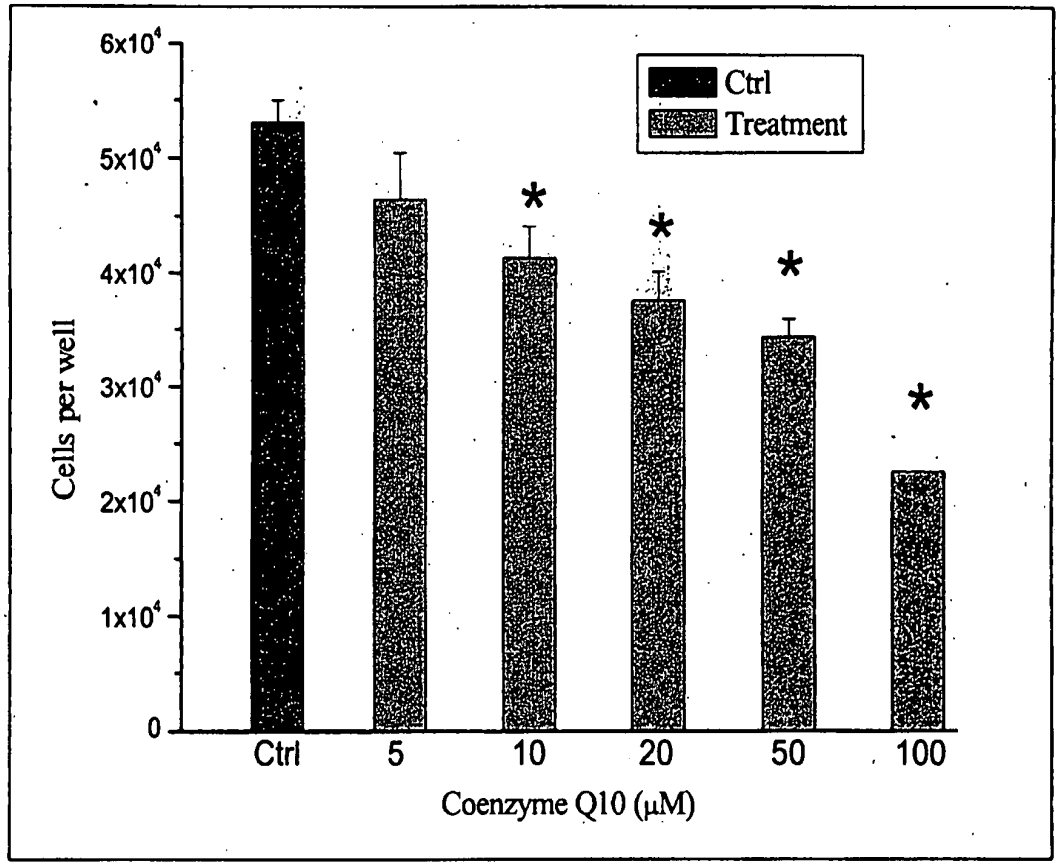


FIG. 1

The Effect of Coenzyme Q10 on Human Melanoma cell line SKMEL28

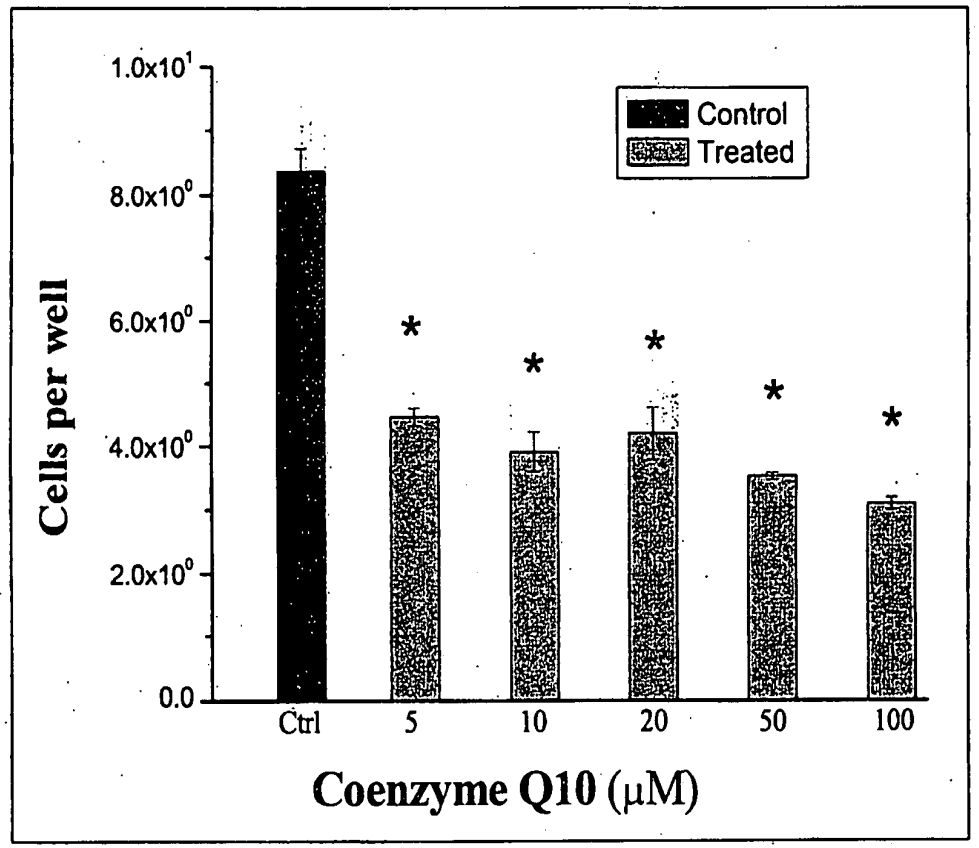


After 36hr Incubation

* *Significant compared to Ctrl* ***P<0.05***

FIG. 2

The Effect of Coenzyme Q10 on Human Melanoma cell line SKMEL28

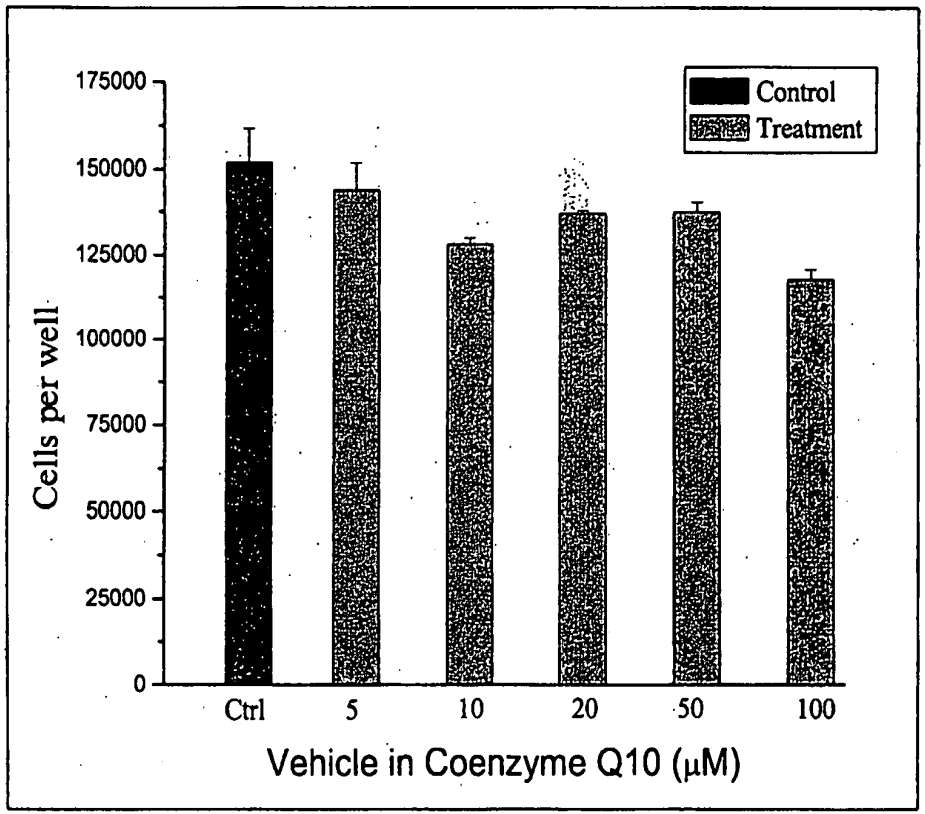


After 48hr Incubation

* *Significant compared to Ctrl* ***P<0.05***

FIG. 3

The Effect of Coenzyme Q10 Vehicle on Human Melanoma cell line SKMEL28



After 48hr Incubation

* *Significant compared to Ctrl* ***P<0.05***

FIG. 4

Comparison of the Effect of Coenzyme Q10 on Apoptosis between Human Melanoma and Fibroblasts cells

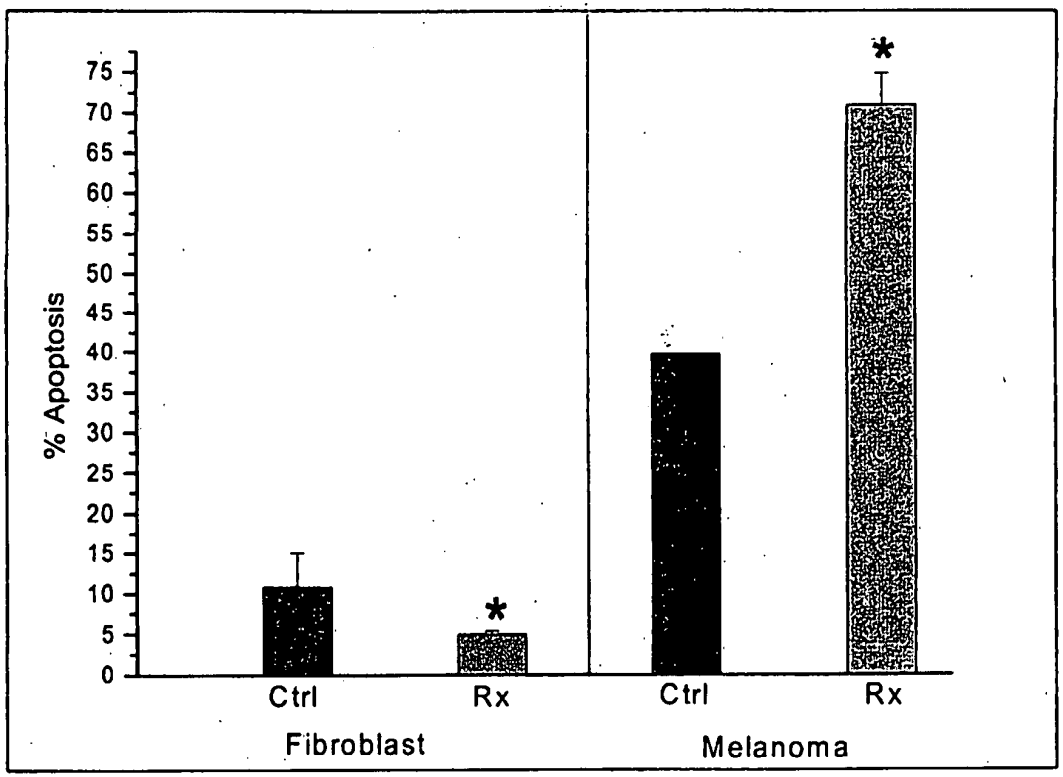
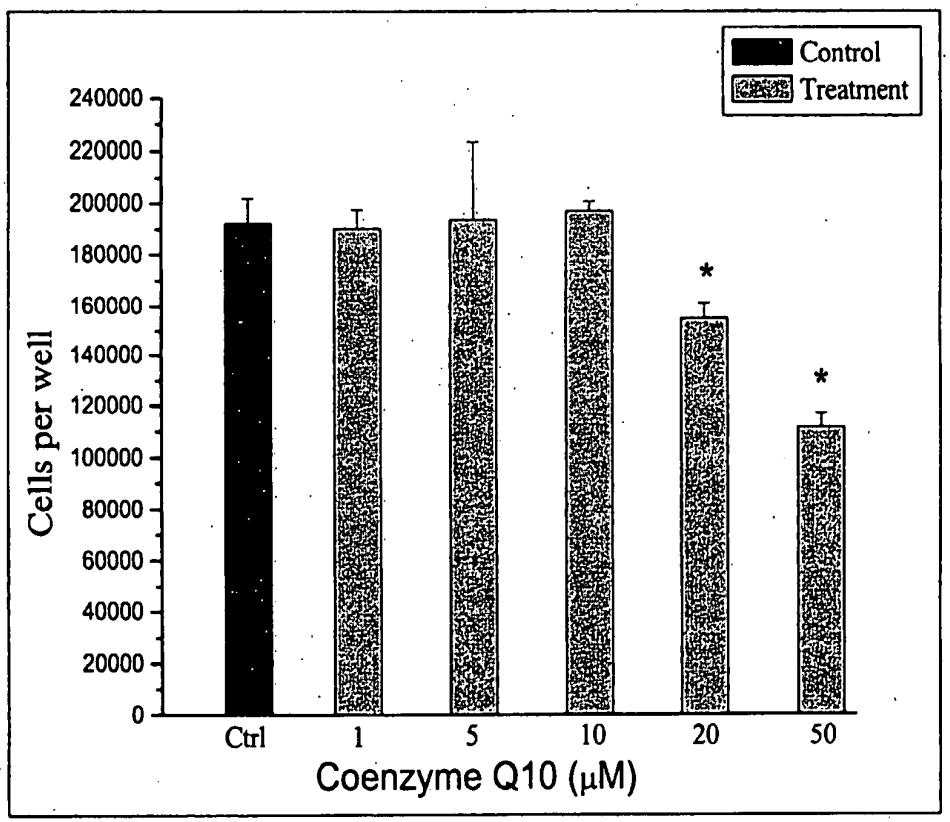


FIG. 5

The Effect of Coenzyme Q10 on Squamous Carcinoma Cells

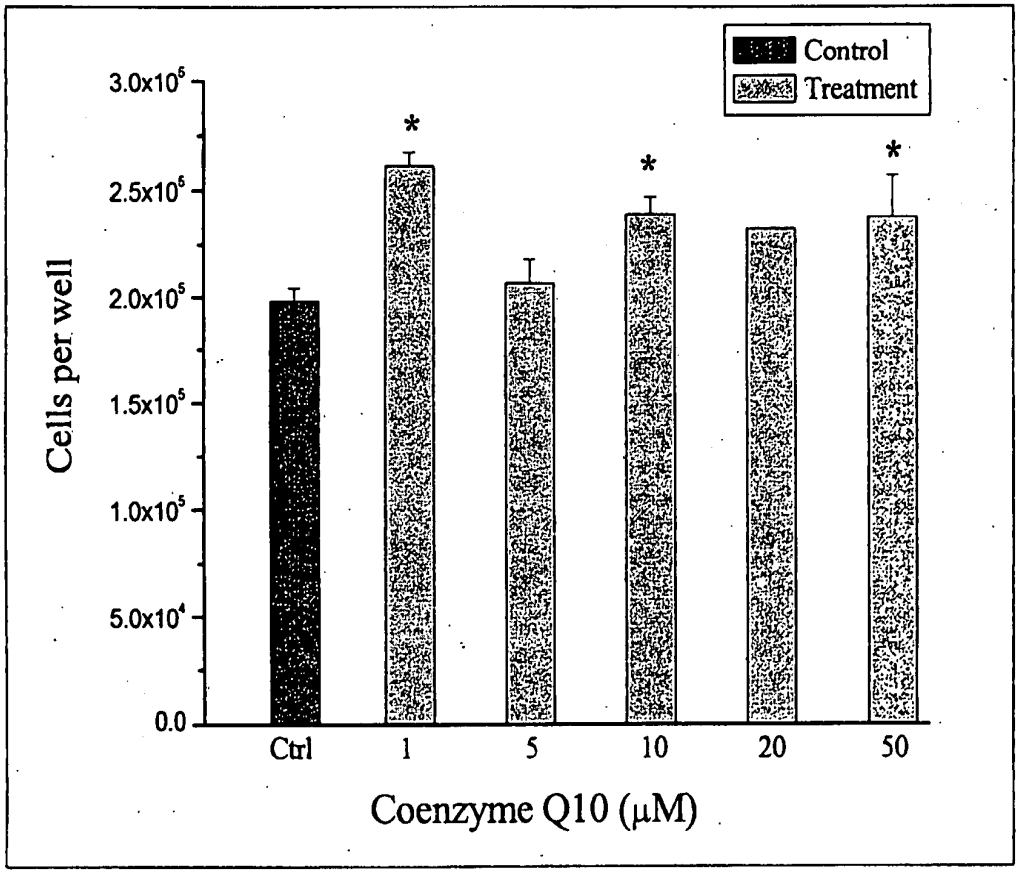


After 48hr Incubation

* *Significant compared to Ctrl* ***P<0.05***

FIG. 6

The Effect of Coenzyme Q10 on Human Neonatal Fibroblast

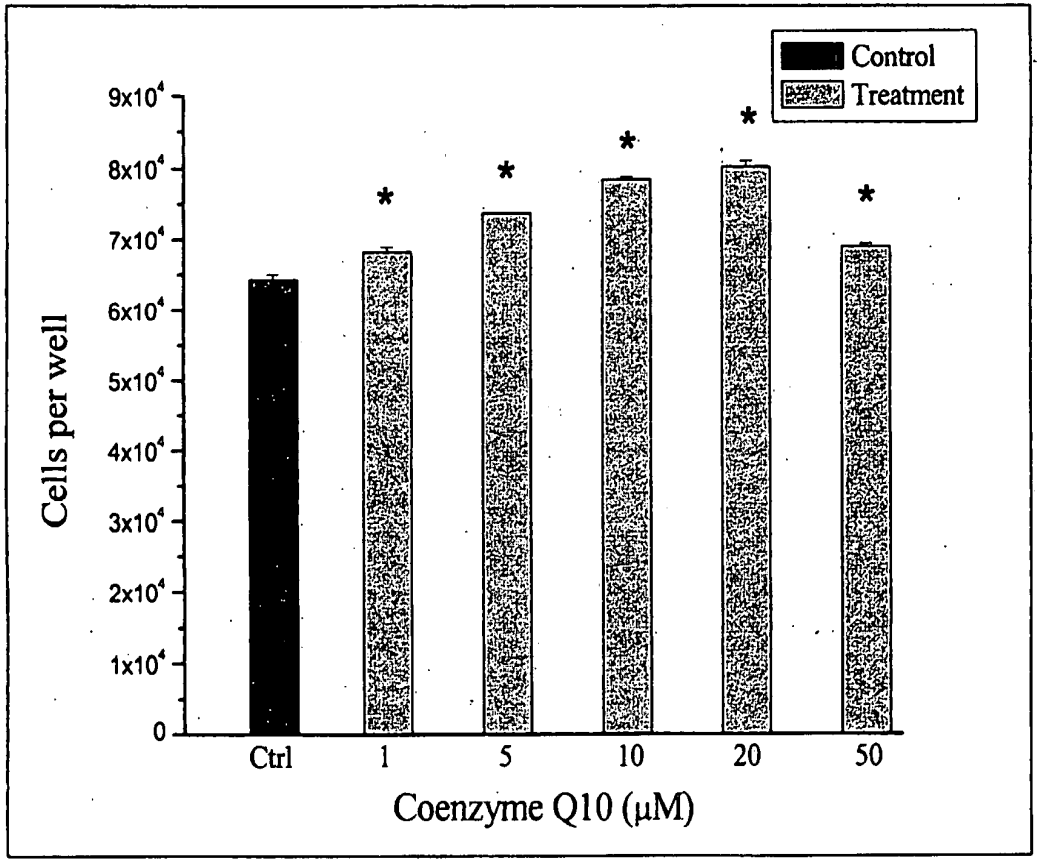


After 48hr Incubation

* *Significant compared to Ctrl* ***P<0.05***

FIG. 7

The Effect of Coenzyme Q10 on Human Neonatal Keratinocytes

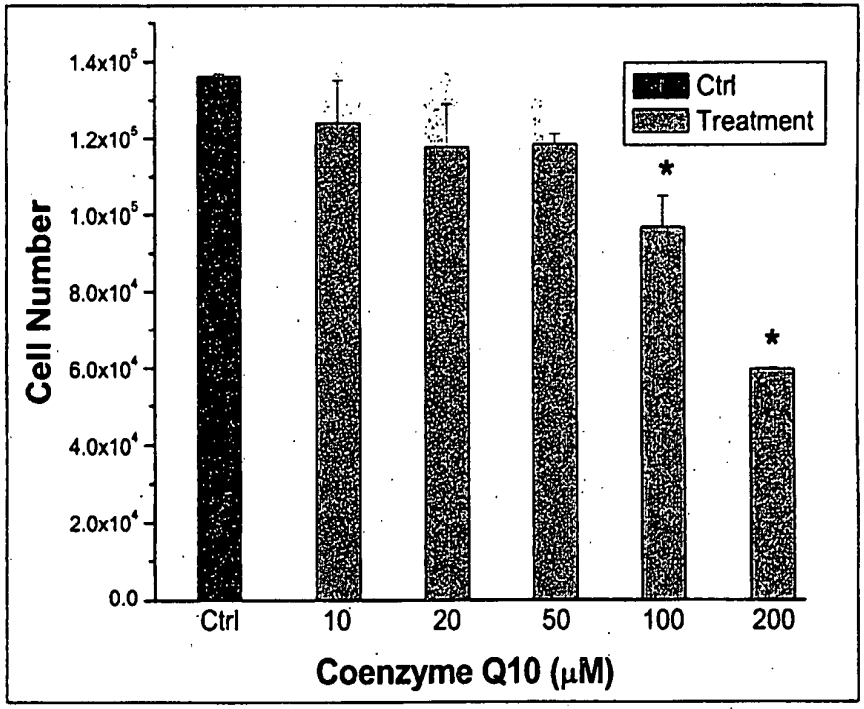


After 48hr Incubation

* *Significant compared to Ctrl* ***P<0.05***

FIG. 8

The Effect of Rx The Effect of Coenzyme Q10 on Human Breast Adenocarcinoma MCF-7 Cell line

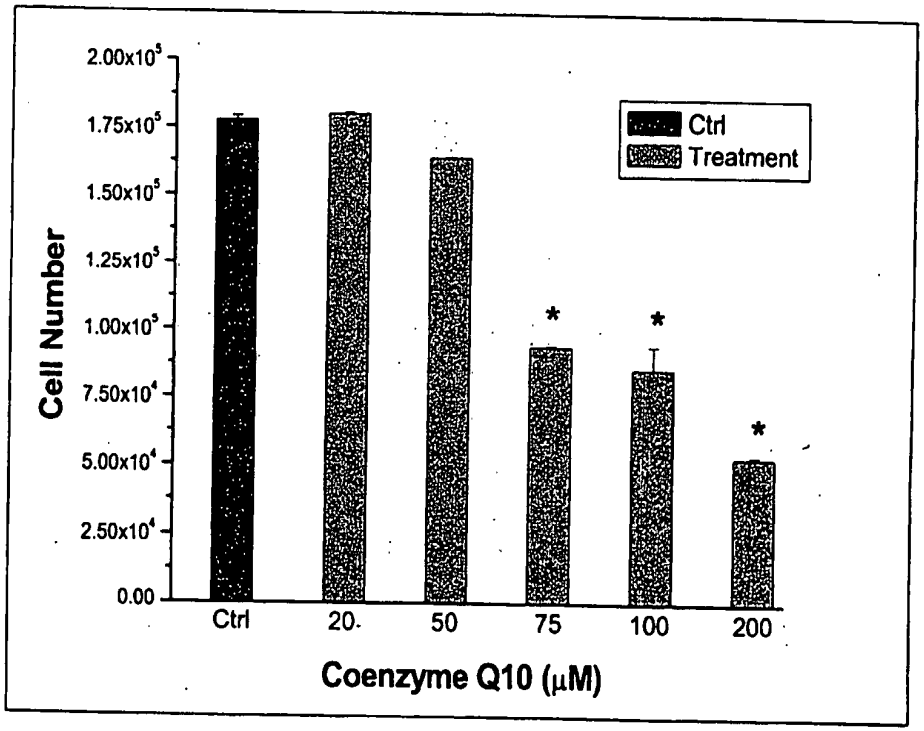


After 48hr Incubation

* *Significant compared to Ctrl* ***P<0.05***

FIG. 9

The Effect of Rx The Effect of Coenzyme Q10 on Human Breast Adenocarcinoma MCF-7 Cell line



After 72hr Incubation

* *Significant compared to Ctrl* ***P<0.05***

FIG. 10

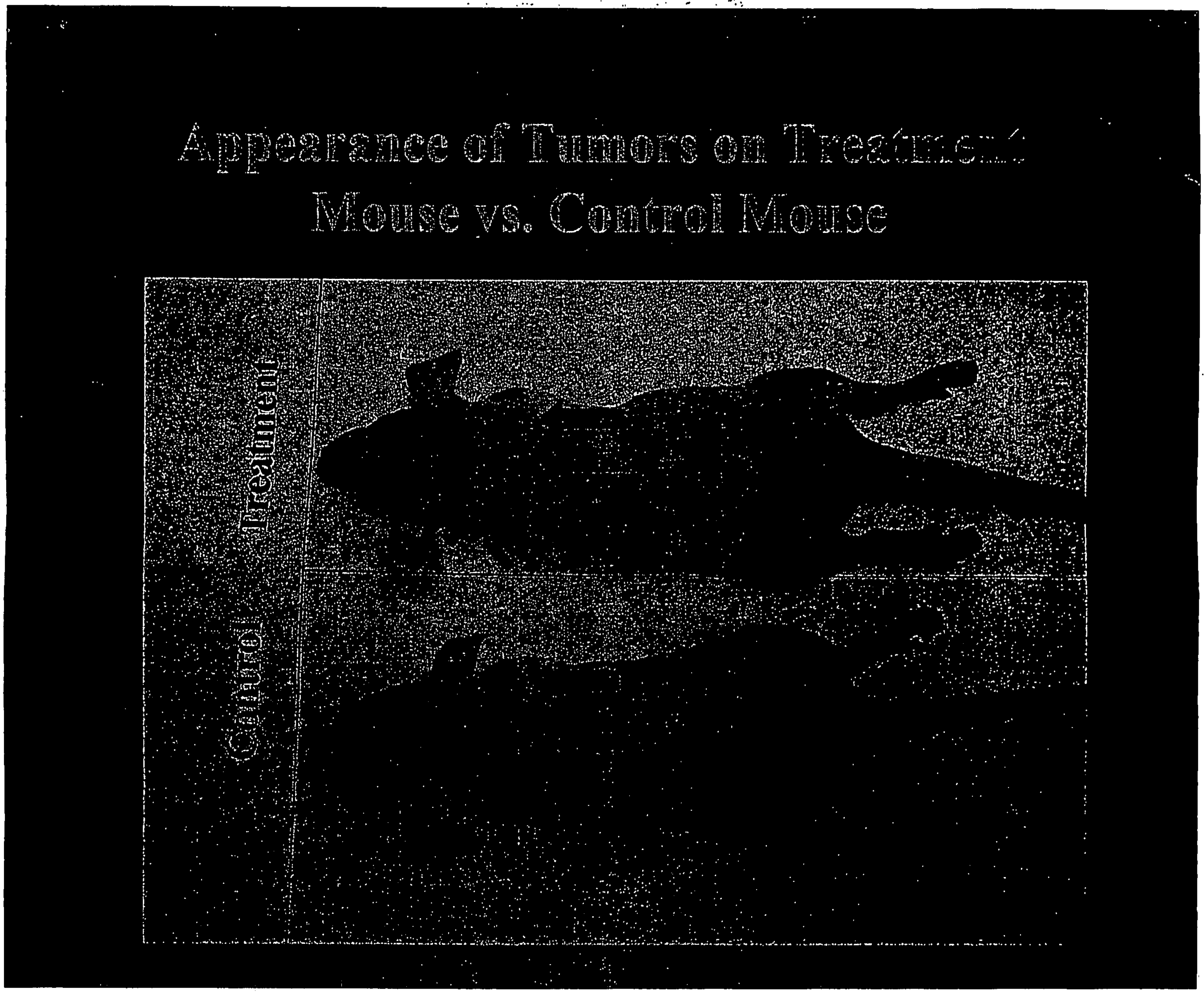


FIG. 11

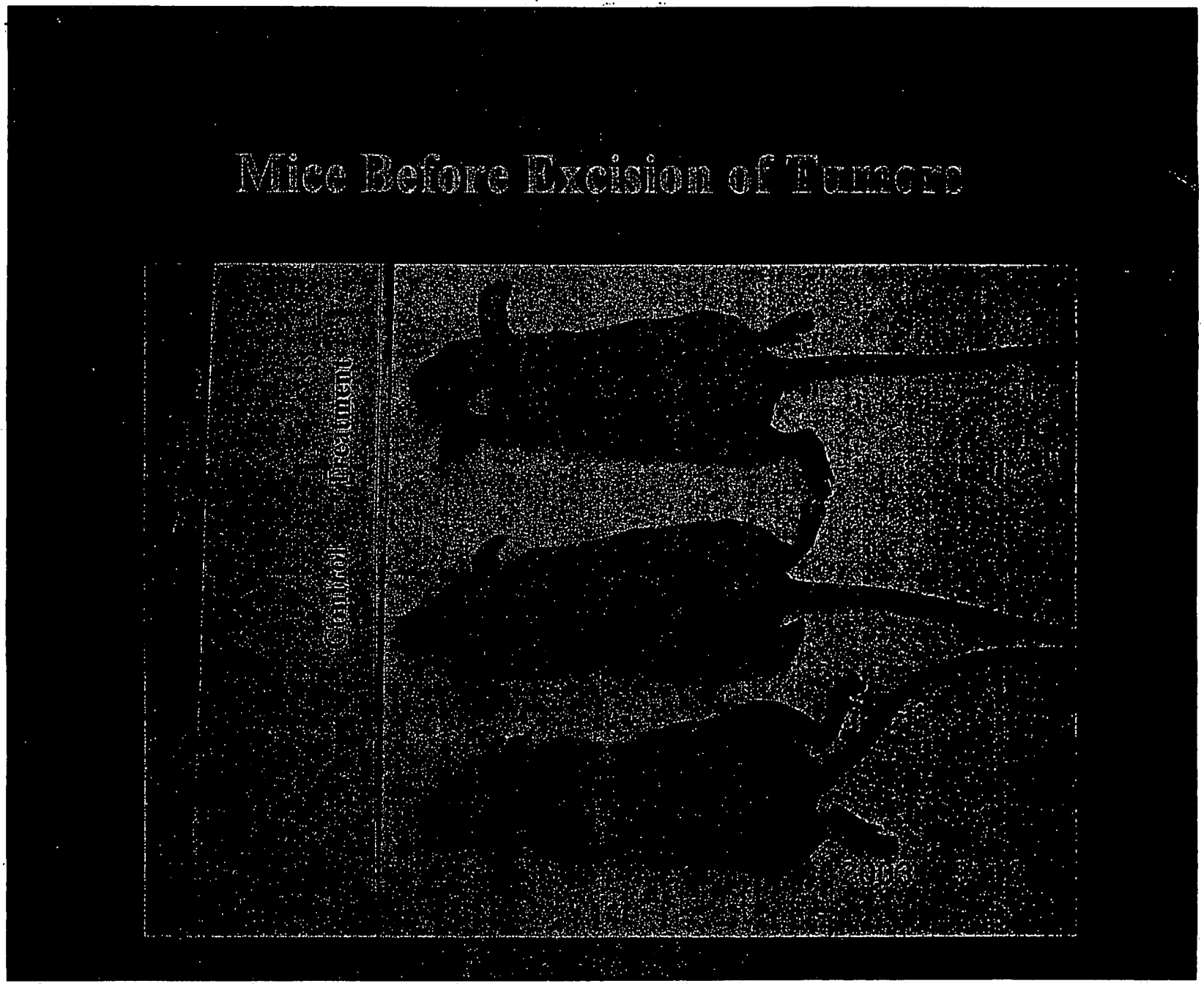


FIG. 12

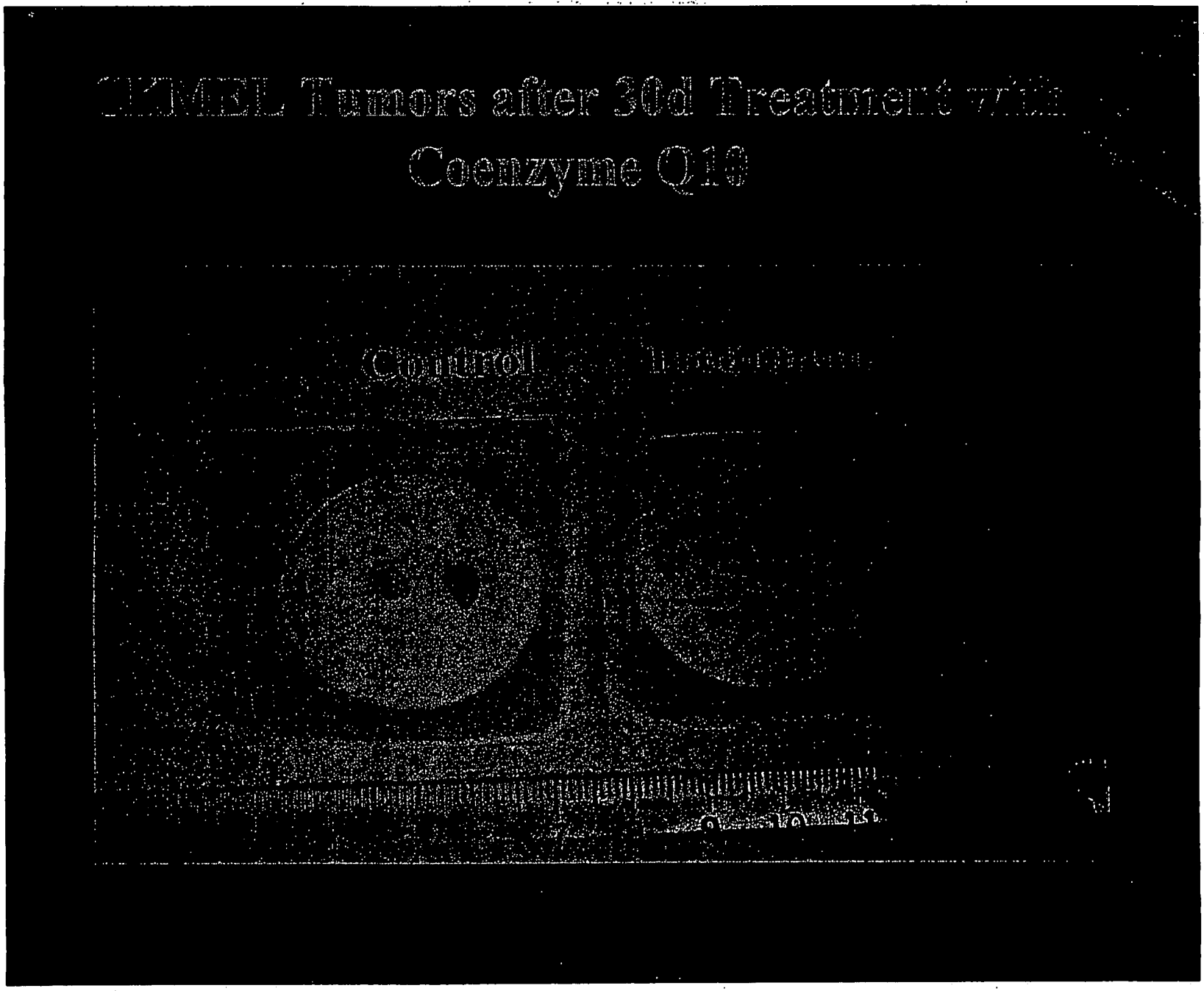
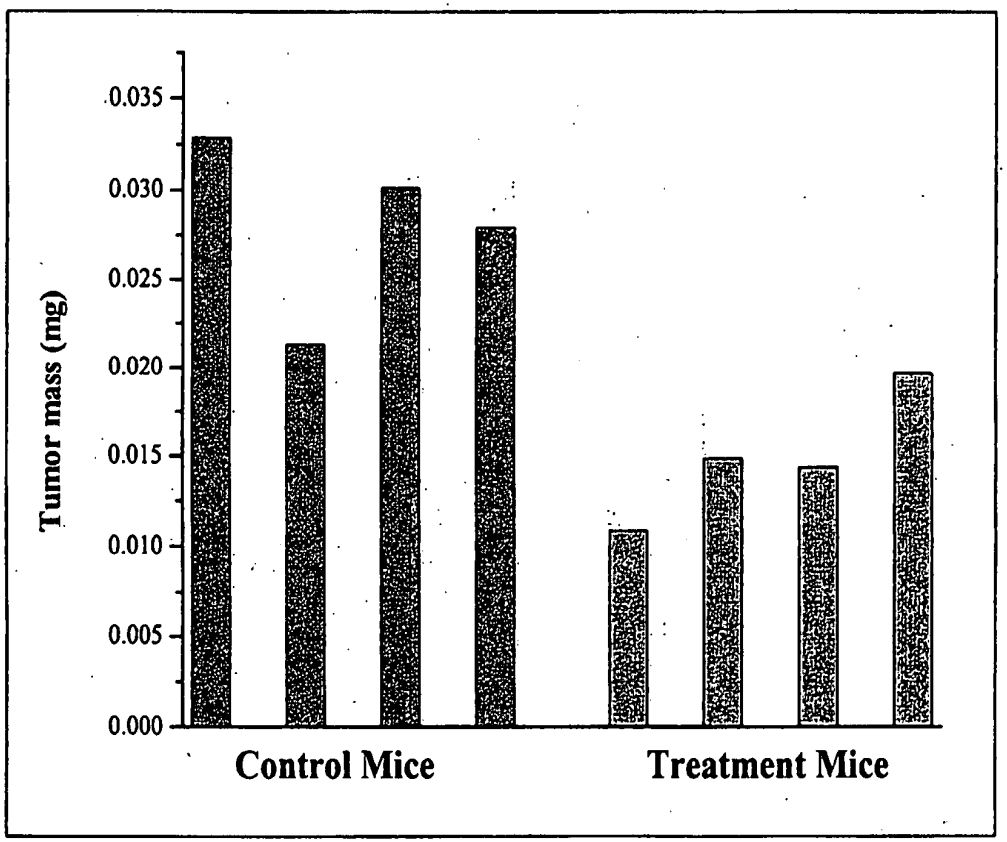


FIG. 13

The Effect of a Topical Formulation of Coenzyme Q10 on SK-28 Tumor Mass in Mice



After 30 days

FIG. 14